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EVIDENCE OF *BRUCELLA* SP. INFECTION IN MARINE MAMMALS STRANDED ALONG THE COAST OF SOUTHERN NEW ENGLAND

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Abstract: After recent isolations of *Brucella* sp. from pinnipeds and cetaceans, a survey was initiated to investigate the prevalence of *Brucella* sp. infections and serologic evidence of exposure in marine mammals stranded along the coasts of Connecticut and Rhode Island. One hundred and nineteen serum samples from four species of cetaceans and four species of pinnipeds were collected from 1985 to 2000 and tested for antibodies to *Brucella* sp. using the brucellosis card test, buffered acidified plate antigen test, and rivanol test. In addition, 20 of these were necropsied between 1998 and 2000, with lymphoid and visceral tissues cultured for *Brucella* sp. Three of 21 (14%) harbor seals (*Phoca vitulina*) and four of 53 (8%) harp seals (*Phoca groenlandica*) were seropositive. *Brucella* sp. was isolated from two of four (50%) harbor seals and three of nine (33%) harp seals. Of the five animals with positive cultures, two were seropositive and three seronegative. *Brucella* sp. was most frequently cultured from the lung and axillary, inguinal, and prescapular lymph nodes. Tissues from which *Brucella* sp. was isolated showed no gross or histopathologic changes. These results indicate that marine mammals stranded along the coast of southern New England can be exposed to and infected with *Brucella* sp.

Key words: *Phoca vitulina*, *Brucella* sp., *Phoca groenlandica*, seal, marine mammal, stranding.

INTRODUCTION

Brucella sp. can infect many domestic terrestrial mammals and wildlife species such as bison (*Bison bison*), elk (*Cervus elaphus*), wild boars (*Sus scrofa*), foxes (*Vulpes* spp.), hares (*Lepus* spp.), and caribou (*Rangifer tarandus*).⁷ Recently, *Brucella* sp. has been demonstrated by culture and serology in a number of marine mammal species worldwide. These include Atlantic white-sided dolphin (*Lagenorhynchus acutus*),¹² bottlenose dolphin (*Tursiops truncatus*),⁸ common dolphin (*Delphinus delphinus*),¹⁹ striped dolphin (*Stenella coeruleoalba*),⁹ harbor porpoise (*Phocoena phocoena*),¹⁷ fin whale (*Balaenoptera physalus*),²⁰ minke whale (*Balaenoptera acutorostrata*),²⁰ sei whale (*Balaenoptera borealis*),²⁰ harbor seal (*Phoca vitulina*),¹⁰ hooded seal (*Cystophora cristata*),¹² grey seal (*Halichoerus grypus*),¹³ ringed seal (*Phoca hispida*),¹⁵ and Atlantic walrus (*Odobenus rosmarus*).¹⁵ The bacteria have been isolated from a variety of tissues, in-

cluding spleen, mammary gland, regional lymph nodes, and s.c. adnexa.¹⁹ On the basis of biochemical tests and 16S ribosomal ribonucleic acid sequence analysis, *Brucella* sp. isolated from marine mammals are distinct from those commonly isolated from terrestrial mammals, specifically *B. abortus*, *B. melitensis*, and *B. ovis*.⁴ Brucellosis may adversely affect reproduction in cetaceans, as it does in terrestrial mammals.¹⁴

Marine mammal management policies and practices are often controversial. Seal populations have increased markedly after passing of the Marine Mammal Protection Act of 1972 in the United States, and natural ranges of certain species now seasonally include the entire northeastern coast of the United States.^{11,18} As a result of this expansion, beached marine mammals and humans interact more frequently, and zoonotic disease transmission is of some concern now.

Brucellosis is an economically significant disease,⁶ and it is also a significant zoonosis that is reportable at the national level in the United States.⁵ Although *B. melitensis* remains the principal cause of human brucellosis, *B. abortus*, *B. suis*, and *B. canis* also can infect humans.²¹ One laboratory-acquired human infection suggests that *Brucella* sp. isolated from marine mammals are also pathogenic for humans.³

Considerable efforts have been invested in eradicating brucellosis from livestock,²¹ and intense screening for the disease is routine and necessary to maintain disease-free herds. Screening programs do not exist for marine mammals, and the geo-

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graphic distribution and prevalence of *Brucella* sp. in marine mammals is only now being investigated.

Serologic and bacteriologic evidence of *Brucella* sp. has been reported in marine mammals from the North Atlantic Ocean^{16,20} but not for the southern New England populations. We studied the seroprevalence of *Brucella* sp. in archived sera and sera of recently stranded marine mammals recovered from the Connecticut and Rhode Island coastlines and the prevalence and tissue distribution of *Brucella* sp. infection in stranded animals.

MATERIALS AND METHODS

Animals

Mystic Aquarium, in Mystic, Connecticut, provided all tissue and serum samples from marine mammals stranded along the Connecticut and Rhode Island coasts. Serum samples were obtained from four species of pinnipeds ($n = 108$) and four species of cetaceans ($n = 11$). These included 21 harbor seals, 53 harp seals (*Phoca groenlandica*), 16 hooded seals, 18 grey seals, five Atlantic white-sided dolphins, three common dolphins, a harbor porpoise, and two pilot whales (*Globicephala macrorhynchus*). In addition, three species of pinnipeds ($n = 16$) and two species of cetaceans ($n = 4$) were necropsied and sampled for evidence of *Brucella* sp. infection. These included four harbor seals, nine harp seals, three hooded seals, three common dolphins, and a striped dolphin. All necropsied marine mammals were collected as stranded animals by Mystic Aquarium under authorization from the National Marine Fisheries Service and were collected dead, or had died during rehabilitation, or were euthanatized for humane reasons as judged by an attending veterinarian.

Serology

One hundred nineteen serum samples collected after January 1985 were tested for the presence of antibodies cross-reactive to *B. abortus*. The majority of serum samples were obtained either at the stranding site or on arrival at Mystic Aquarium before any rehabilitation or medical treatments. Diagnostic procedures included the brucellosis card test, buffered acidified plate antigen test (BAPA), and the rivanol test and followed established protocols.²

Although there are no official interpretation guidelines for classifying sera of marine mammals as positive, suspect, or negative when using tests developed for cattle, established protocols were followed.¹⁷ When all three screening tests produced negative results, animals were classified as “nega-

tive,” when one or more, but not all the screening tests were positive, animals were classified as “suspect,” and when all screening tests were positive, animals were classified as “positive.” National Veterinary Services Laboratory, Brucella Reference Laboratory (1800 Dayton Road, Ames, Iowa 50010, USA) supplied positive and negative bovine control sera, which were included with each test.

Bacteriology

A routine set of gross tissues as well as gross lesions was collected from each animal for culture¹ and histologic examination. Tissues not cultured immediately were held at -70°C . Organisms suspected of being *Brucella* sp. on the basis of colony appearance were counted, recorded, and submitted to the National Veterinary Services Laboratory, where further examination using standard techniques of identification was conducted.¹

Histopathology and immunohistochemical technique

The necropsy tissues collected for histologic examination were fixed in 10% neutral-buffered formalin, trimmed to fit plastic cassettes, routinely processed for paraffin embedding, sectioned at $4\text{ }\mu\text{m}$, stained with hematoxylin and eosin, and examined by light microscopy. Tissues from which *Brucella* sp. was isolated were also examined by immunohistochemistry techniques that used a commercially available alkaline phosphatase system (Vector Laboratories, Inc., 30 Ingold Road, Burlingame, California 94010, USA). Tissues sectioned at $4\text{ }\mu\text{m}$ were mounted on positively charged slides, deparaffinized, hydrated to buffer (pH 7.6), blocked for 20 min at 37°C with nonimmune goat serum, and incubated overnight at 4°C with a primary polyclonal antibody. Three primary polyclonal antibodies were used individually and at three different dilutions (1:5,000, 1:1,000, 1:500) for each slide tested. The first primary antibody used was obtained from rabbits immunized with *B. abortus* strain 2308. The second primary antibody was obtained from rabbits immunized with *B. melitensis* strain 16 M. The third primary antibody used was obtained from rabbits immunized with a strain of *Brucella* sp. isolated from a United States West Coast dolphin (Allen E. Jensen, pers. comm.). Slides were then rinsed in buffer for 10 min at 37°C and incubated with a biotinylated goat anti-rabbit IgG secondary antibody for 20 min at 37°C . Slides were rinsed in buffer for 20 min at 37°C , alkaline phosphatase-labeled streptavidin (Vector Laboratories, Inc.,) was applied for 20 min at 37°C , slides were rinsed in buffer for 10 min, and alkaline phos-

Table 1. Results of *Brucella* serology survey for marine mammals stranded along the coasts of southern New England from 1985–2000.

Species	Number	Positive (%)	Negative (%)	Suspect (%)
<i>Lagenorhynchus acutus</i>	5	0	5 (100)	0
<i>Delphinus delphinus</i>	3	0	3 (100)	0
<i>Phocoena phocoena</i>	1	0	1 (100)	0
<i>Globicephala macrorhynchus</i>	2	0	2 (100)	0
<i>Phoca vitulina</i> ^a	21	3 (14)	7 (33)	11 (53)
<i>Phoca groenlandica</i> ^b	53	4 (8)	23 (43)	26 (49)
<i>Cystophora cristata</i>	16	0	7 (44)	9 (56)
<i>Halichoerus grypus</i>	18	0	8 (44)	10 (56)
Total	119	7 (6)	55 (46)	57 (48)

^a Positive samples dated from 1988, 1992, and 1996.

^b Positive samples dated from 1999 (3), and 2000.

phate substrate was applied and rinsed off with buffer after 5 min at 37°C. Slides were then counterstained with Nuclear Fast Red, dehydrated in Propar (xylene-substitute, Anatech Ltd., 6621-F Electronic Drive, Springfield, Virginia 22151, USA), and coverslips were mounted with Permount. Positive and negative controls were known *Brucella* sp. infected and uninfected bison placenta, respectively. Normal rabbit serum was substituted for the primary antibody and used as an additional negative control.

RESULTS

Serology

Seven of 119 (6%) serum samples had antibodies to *Brucella* sp. Positive results were recorded for three of 21 (14%) harbor seals and four of 53 (8%) harp seals (Table 1). Of the sera tested, the earliest evidence of exposure to *Brucella* sp. was detected in a harbor seal sample drawn in 1988 (Table 1). None of the cetaceans tested seropositive. A large number of animals (48%), almost all pinnipeds, tested positive for one or two of three serology tests resulting in a suspect diagnosis. No apparent sex predilection was noted in seropositive seals. However, most seropositive animals were less than 1 yr of age.

Bacteriology

Brucella sp. was isolated from tissues of five of 20 (25%) animals examined, including two of four (50%) juvenile harbor seals and three of nine (33%) juvenile harp seals. The tissues from which *Brucella* sp. was isolated included lung and axillary, cervical, colorectal, gastric, hepatic, inguinal, mediastinal, mesenteric, pelvic, prescapular, pulmonary, sublingual, sublumbar, and submandibular lymph nodes (Table 2).

Histopathology and immunohistochemical technique

Gross and histopathologic examination of tissues from infected animals revealed no pathologic changes associated with *Brucella* sp. infection. Immunohistochemistry techniques labeled *Brucella* sp. within the gut lumen and uterus of several lungworms (*Paraflaroides* sp.) from lung sections of one seal. In 1997, similar findings were reported from a different *Paraflaroides* sp.¹⁰ No positive antigen labeling was produced in any of the tissues from which *Brucella* sp. was isolated.

DISCUSSION

In 1994, evidence of *Brucella* sp. infection in marine mammals was first reported in a bottlenose dolphin.⁸ Since then, species of *Brucella* have been isolated from marine mammals found stranded along the coast of Scotland,¹² inhabiting the North Atlantic Ocean²⁰ and the Pacific Ocean.¹⁰ There has also been serologic evidence of *Brucella* sp. exposure in Atlantic walruses and ringed seals from the Canadian Arctic,¹⁵ in many species of North American pinnipeds and cetaceans,¹⁶ and in marine mammals found stranded along the coast of England and Wales.¹³

Our serologic and bacteriologic results indicate that marine mammals stranded along the coasts of southern New England have been exposed to, and infected with, *Brucella* sp. Because serology provides evidence of previous exposure and not necessarily active, ongoing infection, postmortem examinations were performed to determine the tissue distribution of *Brucella* sp. infection in stranded marine mammals that did not respond to rehabilitation efforts.

Brucella sp. was isolated from five seals of the 20 (25%) marine mammals collected and examined

Table 2. Results of *Brucella* serology and bacteriology survey for marine mammals stranded along the coasts of southern New England from 1998–2000.^a

Species	ID	Sex	Serology	Bacteriology	Positive tissues
<i>Delphinus delphis</i>	00-298-1	F	NSA	—	
<i>Delphinus delphis</i>	00-298-2	M	NSA	—	
<i>Delphinus delphis</i>	00-298-2	M	NSA	—	
<i>Stenella coeruleoalba</i>	00-394	M	NSA	—	
<i>Phoca vitulina</i>	98-7992	F	S	—	
<i>Phoca vitulina</i>	00-1845-1	F	NSA	—	
<i>Phoca vitulina</i>	00-1845-2	M	—	+	1,2,5,7,9,12
<i>Phoca vitulina</i>	00-1845-3	M	—	+	2
<i>Phoca groenlandica</i>	00-1468	F	NSA	—	
<i>Phoca groenlandica</i>	00-1846	M	NSA	—	
<i>Phoca groenlandica</i>	99-605	M	—	+	1,2,3,8
<i>Phoca groenlandica</i>	99-934	M	+	+	2,4,6,7,11,14,15
<i>Phoca groenlandica</i>	99-1583	M	NSA	—	
<i>Phoca groenlandica</i>	99-2668	M	S	—	
<i>Phoca groenlandica</i>	00-193	M	S	—	
<i>Phoca groenlandica</i>	00-1035	M	NSA	—	
<i>Phoca groenlandica</i>	00-1055	F	+	+	10,11,13
<i>Cystophora cristata</i>	99-5926	M	—	—	
<i>Cystophora cristata</i>	00-1141	F	NSA	—	
<i>Cystophora cristata</i>	00-1142	M	NSA	—	

^a 1, Lung; 2, Axillary lymph node; 3, Cervical lymph node; 4, Colorectal lymph node; 5, Gastric lymph node; 6, Hepatic lymph node; 7, Inguinal lymph node; 8, Mediastinal lymph node; 9, Mesenteric lymph node; 10, Pelvic lymph node; 11, Prescapular lymph node; 12, Pulmonary lymph node; 13, Sublingual lymph node; 14, Sublumbar lymph node; 15, Submandibular lymph node; NSA, No serum available; S, Suspect; +, Positive; —, Negative.

during 1998–2000. Of these five animals, two were seropositive and three were seronegative. One possible explanation for an infected animal to test seronegative is that antibody concentrations associated with very recent infection are too low to be detected with the tests used. *Brucella* sp. could not be cultured from any of the cetaceans examined, so cetaceans may be less susceptible than pinnipeds to *Brucella* sp. infection.

The serology tests used in this study, specifically the card, BAPA, and rivanol, were the only tests available. Buffered *Brucella* antigen tests, including the card test, have been accepted as efficient for use in both cattle and swine, suggesting cross-reactivity between *B. bovis* and *B. suis*.² It is possible that the serology tests used in this study also show cross-reactivity with the *Brucella* sp. identified in marine mammals.

A recent serologic survey used two competitive enzyme-linked immunosorbent assays using monoclonal antibodies specific to *B. abortus* cell wall components.¹⁶ Although these tests may also be effective in the identification of *Brucella* sp. in marine mammals, a study comparing all these tests should be conducted. More information is needed to validate the serologic tests used in this and other

surveys because all the tests were developed for cattle and cross-reactions between *Brucella* sp. and other gram-negative organisms have been reported.¹⁷

Nine of the 20 animals collected during 1998–2000 were evaluated by both serology and microbiologic culture of tissues (Table 2). Given this small number, it is difficult to draw definite conclusions on the correlation between the serology and bacteriology results. These results appear useful in determining which marine mammal tissues are best for isolating *Brucella* sp. These include lung and axillary, inguinal, and prescapular lymph nodes from which *Brucella* sp. was cultured more than once (Table 2). Although *Brucella* sp. was also recovered from cervical, colorectal, gastric, hepatic, mediastinal, mesenteric, pelvic, pulmonary, sublingual, sublumbar, and submandibular lymph nodes, all these tissues were not consistently sampled from each animal.

The tissue distribution of *Brucella* sp. in marine mammals was similar to that previously reported for terrestrial mammals.²¹ Although there is serologic and bacteriologic evidence of exposure and infection in numerous species, it is not known whether *Brucella* sp. causes disease in all marine

mammals similar to that seen in terrestrial mammals. Brucellosis does adversely affect reproduction in cetaceans because two adult bottlenose dolphins with placentitis and fetuses presumably aborted as a result of *Brucella* infection.¹⁴ *Brucella* sp. could not be isolated from the reproductive tracts of any culture-positive animal in this study. Although it has not been determined whether host sexual maturity or pregnancy plays a role in *Brucella* sp. infection in marine mammals as it does in terrestrial mammals, it is possible that *Brucella* sp. did not localize in the juvenile reproductive tracts of these subjects.

The finding of *Brucella* sp. within the gut lumen and uterus of several lungworms from the lung sections of one seal suggests that infected lungworms may play a role in maintaining and transmitting brucellosis among marine mammals.¹⁰

Further studies of the prevalence and pathogenicity of *Brucella* in marine mammals are necessary to fully understand the significance of *Brucella* infection in marine mammals and the potential for its transmission to other species.

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